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The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

01200228.3

Der Präsident des Europäischen Patentamts;  
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets  
p.o.

R C van Dijk

DEN HAAG, DEN  
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**Blatt 2 d r Bescheinigung**  
**Sheet 2 of the certificate**  
**Page 2 de l'attestation**

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Demandeur(s):  
Amsterdam Support Diagnostics B.V.  
1105 BA Amsterdam  
NETHERLANDS

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23.01.2001

Title: Means and methods for treatment evaluation.

(100)

The invention relates to the field of medicine. The invention particularly relates to the fields of molecular biology and detection methods.

Recent advances in the knowledge of molecular processes in a cell  
5 and techniques to study these processes have resulted in improved methods of  
typing and treating diseases. Understanding of the underlying molecular  
diversity of tumors has, for instance, already led to a better understanding of  
the diversity of response to treatment of morphologically similar tumors.  
Improved typing influences the way tumor patients are being treated. A  
10 drawback of the current methods of treatment is, however, that it takes a  
relatively long time to determine whether a treatment given to a patient is  
actually effective. This impedes the optimization of dosages and/or schedules  
with which treatment is given. Moreover, it also slows down the possibility to  
adjust the treatment regimen all together. For instance, adjustment of  
15 therapy is currently only possible when macroscopic analysis of tumor cells in  
the body indicates that the therapy given is not effective. Macroscopic changes  
typically need several weeks to manifest themselves and equipment to  
measure such changes is often not readily available.

The present invention provides a method for determining whether a  
20 treatment is effective in changing the status of a certain set of target cells in a  
patient comprising obtaining a sample from said patient after initiation of said  
treatment and determining whether said sample comprises an expression  
product of at least one marker gene. Preferably, said set of target cells  
comprises a tumor cell. It was found that tumor cells and/or surrounding  
25 tissue respond, on a molecular level, very quickly to an effective treatment.  
This response can be detected by measuring an expression product of a marker  
gene. Marker gene expression products are indicative for a response to  
treatment. Marker genes are typically genes that are expressed by said set of  
target cells, for instance tumor cells, and/or surrounding tissue. Alternatively,

marker gene expression can be initiated upon treatment given to the patient. Marker gene expression products are responsive to treatment given to a patient. A response can be an alteration in the relative amounts of expression product. However, it can also be an alteration in absolute presence or absence of expressed product such as RNA and/or protein.

According to the invention, a sample which is obtained from a patient may comprise at least one of said target cells. This is particularly suitable for detecting circulating tumor cells which have released themselves from a tumor and are circulating in the blood of a patient. Alternatively, said sample may not comprise any target cells.

With a method of the invention it is possible to determine whether a treatment is effective in said patient. This can be done while a treatment is given or shortly after said treatment. Thus it is possible for instance to adjust treatment schedule, dosages and type on a patient per patient basis. It is preferred that said sample is obtained within a week of initiation of treatment. More preferably, said sample is obtained within two days of initiation of treatment. With a method of the invention it is possible to evaluate treatment effectivity almost immediately after initiation of said treatment. A method of the invention thus offers a good opportunity for determining whether treatment adjustments are required.

A marker gene preferably comprises a gene involved in the generation, maintenance and/or breakdown of blood vessels (angiogenesis). Tumor cells are dependent on the growth of new blood vessels to maintain expansion of tumor mass. On the one hand, blood vessels are required to carry nutrients to the site of the tumor, whereas on the other hand waste material needs to be transported from the tumor. In the present invention it has been shown that expression products from genes involved in the generation, maintenance and breakdown of a blood vessel are among the first to respond to anti-tumor treatments. Such genes are therefor very suitable marker genes of the invention. In one embodiment said marker gene comprises a sequence as

depicted in table 1 or 2. In another embodiment said marker gene comprises a sequence as depicted in figure 1.

A change in the level of expression product of a marker gene is indicative for whether a treatment is effective or not. For instance, the level of expression product of a marker gene can be enhanced in a sample when a treatment is effective, alternatively expression product of a marker gene can be reduced. Thus, preferably, expression product of a marker gene is quantified. The level of expression product in a sample can vary due to changes in the expression of a marker gene. However, it is also possible that the level changes due to a change in type of cells comprising said expression product in said sample, for instance due to treatment related cell death at the site of the body where the sample is obtained. Considering that the level of expression product of marker genes can vary from patient to patient, it is preferred that a method of the invention further comprises comparing the level of expression product of said marker gene with a reference. Preferably said reference comprises the same type of tumor cells prior to, or in the absence of, said treatment. Preferably, said tumor cells are derived from the same patient. The difference in the level of expression product of a marker gene in an effective and a non-effective treatment can be very large. In the extreme cases the level of expression product can range from detectable to not detectable. Marker genes displaying such zero to one relation in expression product levels are preferred in the present invention. A zero to one relation can be used to design relatively simple test systems. A zero to one relation is of course dependent on the detection system used to detect expression product of a marker gene. Very sensitive expression detection systems will typically detect expression product where a less sensitive systems detects no expression product. An expression product can be RNA or a part thereof, transcribed from said marker gene or a translated protein or a part thereof. A person skilled in the art is well capable of designing the most appropriate expression detection system to practice this preferred embodiment of the invention.

A part of an RNA molecule is defined herein as an RNA sequence, comprising at least 50 nucleotides. A part of a protein is defined herein as a part which has at least one same kind of property in kind, not necessarily in amount, as said protein.

5 In a preferred embodiment said tumor comprises Kaposi's Sarcoma.

Kaposi's Sarcoma is a disease of proliferating blood vessels and therefore very much suited for identifying marker genes involved in angiogenesis. According to the invention, changes in angiogenesis factors are among the first marker events as a result of treatment.

10 Kaposi's Sarcoma (KS) manifests itself clinically by reddish skin lesions.

Kaposi's Sarcoma is a multicentric, malignant neoplastic vascular proliferation characterized by the development of bluish-red cutaneous nodules, usually on the lower extremities, most often on the toes or feet, and slowly increasing in size and number and spreading to more proximal areas. The tumors have  
15 endothelium-lined channels and vascular spaces admixed with variably sized aggregates of spindle-shaped cells, and often remain confined to the skin and subcutaneous tissue, but widespread visceral involvement may occur. Kaposi's Sarcoma occurs spontaneously in Jewish and Italian males in Europe and the United States. An aggressive variant in young children is endemic in some  
20 areas of Africa. A third form occurs in about 0.04% of kidney transplant patients. There is also a high incidence in AIDS patients. (From Dorland, 27th ed & Holland et al., Cancer Medicine, 3d ed, pp2105-7).

Kaposi's Sarcoma is aggressive in HIV infected individuals superinfected with human herpes virus HHV8. The angiogenic mechanism causing the lesions  
25 results from the interplay of viral and cellular gene expression and is poorly understood in terms as to which genes are involved and what controls their expression. The angiogenic proliferation in KS involves mechanisms likely to be universal in angiogenesis. The central role of angiogenesis in Kaposi's Sarcoma is clearly illustrated by the French name for this tumor:  
30 angiosarcomatose kaposi. Because of said central role of angiogenesis in



Kaposi's Sarcoma, determination of marker genes involved in angiogenesis is very suitable to determine whether a treatment of Kaposi's Sarcoma is effective.

- 5           In the present invention, gene expression patterns of Kaposi's Sarcoma were examined with a method called serial analysis of gene expression (SAGE) (Velculescu et al. (1995) Science 270; 484-487). This method allows the quantitative and simultaneous analysis of a large number of transcripts. SAGE is based on two principles. First, a short nucleotide
- 10   sequence TAG (10 base pairs) contains sufficient information to uniquely identify a transcript, provided it is isolated from a defined position within the transcript. Second, concatenation of short sequence TAG's allows the efficient analysis of transcript in a serial manner by sequencing of multiple TAG's within a single clone.
- 15   Briefly, in this method a biotinylated oligo (dT) primer is used to synthesize cDNA from mRNA, and after digestion with a restriction enzyme, the most 3' terminus (near the poly-A tail) is isolated. These 3' fragments of cDNA are ligated to linkers and cleaved with a type II restriction enzyme to release short sequence (10bp) of the original cDNA (TAG's). The TAG's are ligated to
- 20   diTAG's and PCR amplified. These di-TAG's are then ligated to form long concatamers, which are cloned and sequenced. In this way, one sequence reaction yields information about the distribution of many different mRNA's. Finally, the calculation of the abundance of different TAG's and the matching of the TAG's in Genbank are done using the necessary computer software.
- 25           In another aspect the invention provides the use of nucleic acid comprising a sequence as depicted in figure 1 and/or table 1 or 2 in an expression detection method. In yet another aspect, the invention provides the use of a proteinaceous molecule capable of specifically binding a protein encoded by a nucleic acid comprising a sequence as depicted in figure 1 and/or
- 30   table 1 or 2, in a detection method. Preferably, said uses are directed toward

determining whether a treatment is effective in changing the status of a certain set of target cells in a patient. More preferably, said uses are directed toward determining whether a treatment is effective in counteracting a tumor in a patient. In one embodiment of the invention, said tumor comprises  
5 Kaposi's Sarcoma.

In yet another aspect the invention provides the use of a nucleic acid comprising a sequence as depicted in table 1 or 2 and/or figure 1 as an indicator for angiogenesis. For instance, a nucleic acid comprising a sequence  
10 as depicted in figure 1 and/or table 1 or 2 can be used as detection marker for the process of angiogenesis in the course of regenerative treatment. Changes in the expression level of the detection marker indicate active growth of blood vessels (i.e. angiogenesis) as was meant to induce with the regenerative treatment course. In a preferred embodiment such application is in the field of  
15 heart and coronary disease aimed at generation of new blood supply to affected organs by means of new blood vessels.

In yet another aspect the invention provides the use of a nucleic acid comprising a sequence as depicted in figure 1 and/or table 1 or 2 as detection  
20 marker for tumor cells. In yet another aspect the invention provides the use of a proteinaceous molecule encoded by a nucleic acid comprising a sequence as depicted in figure 1 and/or table 1 or 2 or a proteinaceous molecule capable of binding a protein encoded by a nucleic acid comprising a sequence as depicted in figure 1 and/or table 1 or 2 as detection marker for tumor cells.

25 With a method of the invention it is possible to detect tumor cells that have released themselves from the tumor and are elsewhere in the body. In a preferred embodiment such detection is performed in the blood of a person detecting circulating tumor cells. These circulating tumor cells can be used for primary identification of presence of a tumor somewhere in the body and also

for identification of the risk of metastasis of the tumor to other places in the body next to the primary location of the body.

Thus, in one aspect the present invention provides a use according to the invention for determining the presence of tumor cells in a patient.

The present invention provides a method for determining the presence of tumor cells in a patient. The method comprises the steps of: (a) obtaining a sample of a body fluid from a patient; (b) detecting the presence of tumor cells in the sample; and (c) identifying the type of tumor cells present in the sample. The method is particularly useful for the early detection of tumor cells in a patient, and for the determination of the risk of metastasis of the tumor to other places in the body.

The method of the present invention can be used for the detection of tumor cells in a patient, and for the determination of the risk of metastasis of the tumor to other places in the body. The method is particularly useful for the early detection of tumor cells in a patient, and for the determination of the risk of metastasis of the tumor to other places in the body.

The method of the present invention can be used for the detection of tumor cells in a patient, and for the determination of the risk of metastasis of the tumor to other places in the body. The method is particularly useful for the early detection of tumor cells in a patient, and for the determination of the risk of metastasis of the tumor to other places in the body.

## Examples

### Example 1

In this example a selection of samples for analysis of expression profiles is  
5 made.

A 31-year old man was demonstrated to be HIV-1 seropositive in February  
1997. The initial CD4 cell count was  $25 \times 10^6/l$ . The patient presented within  
two months a mucocutaneous Herpes simplex infection and an extrapulmonary  
Cryptococcosis for which specific medication was given. The HIV-1 RNA load  
10 at presentation was 15,000 copies/ml and increased to 33,000 copies/ml in  
three months. Then antiretroviral therapy was started with zidovudine,  
lamivudine and indinavir. Immediately after start therapy the HIV-1 RNA  
load dropped below detection limit. In November 1997 the patient presented  
with gradual appearance of an increasing number of violaceous skin lesions  
15 that clinically resembled Kaposi's Sarcoma. The diagnosis was confirmed by  
histological examination of one of the lesions. At start of the chemotherapy  
(bleomycin, vincristine and adriamycine intravenously) KS had progressed to  
about 150 cutaneous lesions. The interval between the courses of  
chemotherapy was three weeks and stopped after the fifth course. Several  
20 lesions had disappeared by three weeks of therapy and complete remission was  
gradually reached after one year.

During chemotherapy several biopsies were taken. The first biopsy was  
obtained 24 hours after start chemotherapy (named KS1) and the second  
biopsy after 48 hours (named KS2). All biopsies were flash-frozen in liquid  
25 nitrogen immediately after surgical removal and stored at  $-80^{\circ}\text{C}$ . Diagnosis of  
Kaposi's Sarcoma was confirmed histopathologically.

Control SAGE libraries KS3 and KS4 were made from frozen material taken at autopsy from two AIDS patients with Kaposi's Sarcoma, both of which died in 1986 without having had any form of chemotherapy or retroviral treatment.

## 5 Example 2

The expression profiles of the biopsy samples were determined using the SAGE technology. All biopsies were cut with a microtome in 15-20  $\mu$ m sections and transferred to a tube containing TRIzol. RNA isolation with TRIzol was performed according to the manufacturer's instructions. Poly (A) RNA was  
10 obtained using the Micro-FastTrack™ 2.0 mRNA Isolation Kit. cDNA preparation and the subsequent steps were performed as described by Velculescu. Primary analysis of the sequence results was performed using software especially designed for SAGE by the Bioinformatics Laboratory of the Academic Medical Centre, Amsterdam (van Kampen *et al.* USAGE: a web-  
15 based approach towards the analysis of SAGE data. Bioinformatics, *in press*). The libraries were also analysed using the Human Transcriptome Map (HTM), a program developed in the AMC, which maps TAG's onto human chromosomes (Caron *et al.* The Human Transcriptome Map reveals a clustering of highly expressed genes in chromosomal domains. Submitted for  
20 publication).

We sequenced ~ 47,000 TAG's from the four biopsies; 47,298 TAG's from KS 1 library, 46,671 from the KS 2 library, 49,335 TAG's from the KS3 library, and 48,814 TAG's from the KS4 library. TAG lists (i.e. individual TAG's plus the  
25 number of appearance) were compared with each other in USAGE, TAG sequences with the highest counts were identified with the amct2g database available in USAGE (with is an improved TAG identification compared with the SAGEmap database from CGAP (available from GenBank). Secondly, TAG lists were mapped to chromosome locations with the HTM program, and at the

same time compared with specific TAG lists (e.g. vascular endothelium, publicly available), and with a compilation of all TAG lists in the SAGEmap database (designated "All" in HTM) TAG's belonging to genes specifically up regulated in KS3 and KS 4 identified (Table 1). Nucleotide 15 was determined  
5 from the original diTAG list in USAGE. The TAG sequence of 15 nt. was checked with GenBank (BLAST) to confirm its identification. A few TAG's were eliminated because of ambiguity in the 15<sup>th</sup> nucleotide, or because of misidentification.

### 10 Example 3

Result of the analysis showing the identifiable TAG's derived from known genes with increased expression in Kaposi's Sarcoma SAGE libraries KS3 and KS4 compared to libraries KS1 and KS2. The TAG numbers are first  
15 normalized to a level of 100.000 total TAG's sequenced per library to allow comparison between the libraries and comparison with other libraries in the public domain.

The sequence catg precedes each TAG sequence given in column 2 of table 1.

Table 1. Overview of identifiable TAG's over-expressed in SAGE libraries KS3 and KS4.

No.	TAG sequence (5' → 3')	Unigene no.	ID	overexpression factor <sup>1</sup>
1.	ccccagtcggc	Hs171596	EphA2	3
2.	cttgacatacc	Hs171695	Dual specificity phosphatase	3
3.	catcacggatc	Hs82112	IL1 receptor, type 1 <sup>2</sup>	10-30
4.	ggccaaaggcc	Hs78436	EphB1	>2
5.	ttgcatatcag	Hs82237	AT group D protein	10 - 15
6.	ccctgttcagc	Hs78824	Tie 1 <sup>2</sup>	2-5
7.	gatcaatcagt	Hs16530	Small ind. cytokine A18	10-20
8.	gagggtgccaa	Hs898	Complement comp. 1Q $\beta$	5-10
9.	taaacctgctg	Hs99923	galectin 7	3-10
10.	gtggccagagg	Hs1420	FGFR3	2-5
11.	tctggcccagc	Hs183	DARC (Duffy blood group)	8-10
12.	caggctcgctac	Hs75066	Translin	2-6
13.	gagcagcgccc	Hs112408	Psoriasin (S100 A7)	> 20 (specific)
14.	acttattatgc	Hs76152	Decorin	2-10
15.	caggcctggcc	Hs74649	Cytochrome C oxydase subunit VIc	2-4
16.	gtgcggaggac	Hs181062	Serum amyloid A1	5-14
17.	acagcggcaat	Hs74316	Desmoplakin	5-10
18.	gatgtgcacga	Hs117729	Keratin 14	10-14
19.	caggtttcata	Hs24395	Small ind. cytokine, B14 (BRAK)	5-10
20.	aactctgaccc	Hs93675	Decidual protein induced by progesterone <sup>2</sup>	3-10

1. TAG numbers of appearance were normalized to library sizes of 100.000 TAG's.

2. Identified as Pan Endothelial Markers by St. Croix *et al.*, Genes expressed in human tumor endothelium.

5 Science 289:1197-1202, 2000.

**Example 4**

Result of the analysis showing the non-identifiable TAG's derived from EST's of genes with unknown function with increased expression in Kaposi's

5 Sarcoma SAGE libraries KS3 and KS4 compared to libraries KS1 and KS2.

The TAG numbers are first normalized to a level of 100.000 total TAG's sequenced per library to allow comparison between the libraries and comparison with other libraries in the public domain.

The sequence catg precedes each TAG sequence given in column 2 of table 2.

10

Table 2. Overview of identifiable TAG's over-expressed in SAGE libraries KS3 and KS4.

No.	TAG sequence (5' → 3')	Unigene no.	ID	X overexpression factor <sup>1</sup>
1.	aaatcaataca	Hs94953	EST	4-10
2.	tggtactggc	Hs108741	EST	4-10
3.	tctgcactgag	Hs173789	EST	2-4
4.	caggctgctgg	Hs60440	EST	4-30
5.	atgacagatgg	Hs13775	EST	5-10
6.	gcacaacaaga	Hs236510	EST	3-10
7.	ccacaggagaa	Hs23579	EST	4-10
8.	ctgtgcggaac	Hs46987	EST	2-10
9.	gatggctgcct	Hs18104	EST	4-20
10.	ctccattgcca	Hs31869	EST	2-10
11.	acctccactgg	Hs112457	EST	Unique <sup>2</sup>

1. TAG numbers of appearance were normalized to library sizes of 100.000 TAG's

2. This TAG does not appear in any other SAGE library than our own libraries and seems to be a unique new indicator gene for angiogenesis.

15



### Example 5

Using an RT-PCR based method we were able to determine that TAG 11 (table 2) indeed represents a differently expressed gene. RNA was isolated from a KS lesion and the first strand cDNA synthesis was primed with an oligo(dT)

5 primer with a 5' M13 tail (5'CTA GTT GTA AAA CGA CGG CCA G-(T)<sub>24</sub> 3').

Ten microliter total RNA was used, plus primer and 5 µl RT-mix (50 mM Tris, pH 8.3, 75mM KCl, 3 mM MgCl<sub>2</sub>, 10 mM DTT), 80 mM dNTPs and 20 units RNAsin were added, followed by an incubation for 3 minutes at 65°C and chilled on ice. The RT reaction starts by adding 5 units AMV RT followed by

10 an incubation of 45 minutes at 42°C. For the PCR we used a 19-base TAG-specific primer (which consisted of 11 nt identified in the sage with a 5' NLAIII restriction site and 5 inosine nucleotides to increase the annealing temperature of the primers) and the -21M13 primer. The RT-mix was added to 80 µl PCR mixture containing the 100 ng of each primers. (-21m13 PRIMER: 5'

15 GTA AAA CGA CGG CCA GT 3' and 5' III IIC ATG ACC TCC ACT GG 3'), 50mM Tris (pH8.3), 20 mM KCl, 0.1 mg BSA per ml, dNTPs (0.1 mM each), 2,4 mM MgCl<sub>2</sub>, and 2 units Taq polymerase. After incubation of 5 minutes at 94°C, the reaction was subjected to 35 cycles of amplification in a thermocycler (9700 Perkin-Elmer). A cycle included denaturation for 1 minute at 95°C, annealing

20 for 1 minute at 55°C and extension for 2 minutes at 72°C. The last cycle was followed by 72°C incubation for 10 minutes.

The amplified fragment was cloned in to an AT plasmid (InvitroGen) and subsequently the insert was sequenced using the dye terminator sequencing

25 kit from Applied Biosystems Inc. The fragment appeared to have a length of 102 base pairs and the sequence analysis of the fragment revealed the sequence as depicted in figure 1. This sequence was identical to an EST sequence identified from human foetal heart (GenBank acc. # AI217565 and

others), which in turn matched a predicted exon on chromosome 19. A relation with angiogenesis has not been described previously and is new.

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... ..  
... ..  
... ..  
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... ..

### Brief description of the drawings

5 Figure 1: Sequence involved in angiogenesis. A change of expression of this sequence after a certain treatment indicates that said treatment is effective. This sequence is identical to an EST sequence identified from human foetal heart (GenBank acc. # AI217565 and others), which in turn matches a predicted exon on chromosome 19. A relation with angiogenesis has not been described previously.

[illegible]

1. The first step in the process of the investigation is the identification of the problem. This is done by the researcher who is interested in the problem. The researcher then identifies the problem and the variables involved in the problem. The researcher then identifies the problem and the variables involved in the problem.

The first of these is the *Journal of the American Medical Association* (JAMA), which has been the most influential of the medical journals in the United States. It was founded in 1883 and has since then published a wide range of medical research, including clinical trials, laboratory studies, and reviews of the literature. The journal is published weekly and is one of the most widely read and cited medical journals in the world.

CLAIMS

1. A method for determining whether a treatment is effective in changing the status of a certain set of target cells in a patient comprising:
  - obtaining a sample from said patient after initiation of said treatment, and
  - determining whether said sample comprises an expression product of at least
- 5 one marker gene.
2. A method according to claim 1, wherein said target cells comprise a tumor cell.
3. A method according to claim 1 or 2, wherein said sample comprises at least one of said target cells.
- 10 4. A method according to any one of claims 1-3, wherein said sample is obtained within a week of initiation of said treatment.
5. A method according to any one of claims 1-4, wherein said sample is obtained within two days of initiation of said treatment.
6. A method according to any one of claims 1-5, wherein said marker gene
- 15 comprises a gene involved in the generation, maintenance and/or breakdown of blood vessels.
7. A method according to any one of claims 1-6, wherein said marker gene comprises a sequence as depicted in table 1 or 2.
8. A method according to any one of claims 1-7, wherein said marker gene
- 20 comprises a sequence as depicted in figure 1.
9. A method according to any one of claims 1-8, wherein expression of said marker gene is quantified.
10. A method according to any one of claims 1-9, further comprising comparing expression of said marker gene with a reference.
- 25 11. A method according to any one of claims 2-10, wherein said tumor comprises Kaposi's Sarcoma.

12. Use of nucleic acid comprising a sequence as depicted in figure 1 and/or table 1 or 2 in an expression product detection method.
13. Use of a proteinaceous molecule capable of specifically binding a protein encoded by a nucleic acid comprising a sequence as depicted in figure 1 and/or
- 5 table 1 or 2, in a detection method.
14. A use according to claim 12 or claim 13, for determining whether a treatment is effective in changing the status of a certain set of target cells in a patient.
15. A use according to any one of claims 12-14, for determining whether a
- 10 treatment is effective in counteracting a tumor in a patient.
16. A use according to claim 15, wherein said tumor comprises Kaposi's Sarcoma.
17. A use of a nucleic acid comprising a sequence as depicted in table 1 or 2 and/or figure 1 as an indicator for angiogenesis.
- 15 18. A use according to claim 12 or claim 13 for determining the presence of tumor cells in a patient.

Title: Means and methods for treatment evaluation.

Abstract

5 The invention provides a method for determining whether a treatment is effective in changing the status of a certain set of target cells, such as a tumor, in a patient. This method implies obtaining a sample from a patient after initiation of a treatment, and determining whether said sample comprises an expression product of at least one marker gene. Said marker  
10 gene may be a gene involved in the generation, maintenance and/or breakdown of blood vessels (angiogenesis). A method of the invention is very suitable to determine within a few days if a certain treatment against Kaposi's Carcinoma is successful. Moreover, this method is suitable for determining the presence of tumor cells in a patient.

15

1. The first part of the document is a letter from the President of the United States to the Congress, dated January 3, 1863. It is a very important document, as it contains the President's message to Congress, and is one of the most important documents in the history of the United States.

2. The second part of the document is a letter from the Secretary of the War Department to the Secretary of the Navy, dated January 3, 1863. It is a very important document, as it contains the Secretary's message to the Secretary, and is one of the most important documents in the history of the United States.

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Figure 1

(100)

5'	10	20	30	40	50
	CATGACCTCC	ACTGGAAGAG	GGGGCTAGCG	TGAGCGCTGA	TTCTCAACCT
	60	70	80	90	100
	ACCATAACTC	TTTCCTGCCT	CAGGAACTCC	AATAAAACAT	TTCCATCCA
	102				
AC	3'				

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